

Genistein modulates gene activity in psoriatic patients

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Despite the impressive advancements in the treatment of psoriasis over the past two decades, there is still a need for further improvement. As previously shown in the literature, genistein (5,7-dihydroxy-3-(4-hydroxyphenyl)chromen-4-one), naturally occurring plant compound displays multidirectional action, also in relation to alleviating psoriasis symptoms. In this work we focused our attention on genistein impact on expression of genes when treating moderate-to-severe psoriasis patients. Testing the effects of this isoflavone on transcript levels in both skin specimens and peripheral blood cells of four psoriatic subjects, we found that this compound modulated activities of genes coding for anti-psoriatic members and anti-inflammatory mediators of inflammation. It impairs the activity of certain genes which are overexpressed in psoriasis, while stimulating the expression of other transcripts that are repressed in dermatosis.

Key words: psoriatic patients; flavonoid genistein; skin biopsy; peripheral blood cells; inflammation

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Abbreviations: ADA, antidrug antibodies; BSA, Body Surface Area; CCL2, C-C motif chemokine ligand 2; CCL4, C-C motif chemokine ligand 4; CCL5, C-C motif chemokine ligand 5; CEBK, Central Register of Clinical Trials; CXCL10, C-XC motif chemokine ligand 10; dbGAP, database of genes associated with psoriasis; DEFB4, human β -defensin 2; FC, fold change; FDA, Food and Drug Administration; FOXO1, transcription factor forkhead box protein O1A; GEN, Genistein; GL, Glyteer; IL1B, interleukin 1B; IL6, interleukin 6; IL8, interleukin 8; NF- κ B, nuclear factor kappa B; PAGD, psoriasis-associated genes database; PASI, Psoriasis Area and Severity Index; PBCs, peripheral blood cells; PGA, Physician's Global Assessment; PI3, peptidase inhibitor 3; PI3K/Akt, phosphoinositide 3 kinase/protein kinase B; PN, psoriatic normal skin; PN_D0, uninvolved psoriatic skin on day 0; PN_D56, uninvolved psoriatic skin on days 56; PP, psoriatic plaque skin; PP_D0, lesional involved psoriatic skin on day 0; PP_D56, lesional involved psoriatic skin on days 56; RPLP0, ribosomal protein lateral stalk subunit P0; SERPINB3, serpin family B member 3; TNF- α , tumor necrosis factor α ; UPL, Universal Probe Library; YWHAZ, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta

do not respond to biological therapy, and some lose the initial response due to the development of antidrug antibodies (ADA) (Hsu *et al.*, 2014). Alternatives are small-molecule drugs and organic compounds targeting the key molecules of selected signalling pathways. Another group of drugs are the so-called biosimilars, which do not show clinically relevant differences in purity, safety and potency relative to a reference therapeutic product. Three of them obtained approval from the Food and Drug Administration (FDA) in 2016; however, further studies are necessary (Gupta *et al.*, 2014; Lee *et al.*, 2013). Treatments used in psoriasis are still of risk of toxicity and adverse reactions. Thus, alternative, safe, and if only possible, natural therapeutic agents are desired.

Bioactive plant-derived metabolites such as flavonoids, have been studied as potential agents in medicine for many years (Molyneux *et al.*, 2007; Nakamura *et al.*, 2010). They are ubiquitous plant secondary products, well-known for their antioxidant and anti-inflammatory properties (Chamcheu *et al.*, 2017; Nijveldt *et al.*, 2001; Pazyar *et al.*, 2015). Among these compounds, genistein (5,7-dihydroxy-3-(4-hydroxyphenyl) chromen-4-one), the major metabolite of soy, is considered as regulator of inflammatory responses in the skin, and therefore can consequently be useful in the treatment of atopic dermatitis, vitiligo and psoriasis (Korkina *et al.*, 2012). Importantly, genistein is a potent inhibitor of the production of pro-inflammatory factors, such as IL1, IL6, and TNF- α by modulating the nuclear factor kappa B (NF- κ B) and phosphoinositide 3 kinase/protein kinase B (PI3K/Akt) pathways in macrophages and endothelial cells. Defining molecular bases of genistein biological action and function in psoriasis is essential to evaluate safety of this composite and to develop finest therapeutic procedures. Therefore, in this study we aimed at determining the pattern of isoflavone genistein impact of on genes' expression in the clinical material, skin specimens and blood samples, derived from psoriatic patients.

MATERIALS AND METHODS

Ethical agreement and patient profile. This multicenter, randomized clinical study was conducted in Poland. Institutional review boards at each study site approved the protocol, and all tested patients provided written informed consent. The investigation was conducted in compliance with the Declaration of Helsinki. The study no. GE/BF/09/13 was registered in the Central Register of Clinical Trials (CEBK) and Independent Ethic Committee under the numbers UR.DBL.BLE.475.0110.2014 and KB/910/13, respectively. Patients were eligible for study enrolment if they were aged 18 years or older with active, clinically stable mild to

INTRODUCTION

Choice of the suitable medical handling depends on the severity of psoriasis. If skin lesions are mild and localised, only topical treatment is applied. At a more advanced stage of psoriasis, systemic therapy is initiated, according to clinical recommendations. Another approach of general treatment is suppressing an excessive immune response by using the selected, classic drugs. Over the past decade, biological drugs have been developed, which are an additional treatment option for moderate and severe psoriasis. Unfortunately, 1/3 of patients

Table 1. Clinical trial design.

		Groups identified for study		
		Transcription analysis		
		Placebo	75 mg GEN	150 mg GEN
		n=1	n=2	n=1
Patient		u.11	u.09	u.12
Age		34	51	60
Sex		male	male	female
Race		caucasian	caucasian	caucasian
Weight (kg)		94	98	67
BMI		29.7	33.5	26.5
PASI	Day 0	7.0	3.6	11.7
	Day 56	8.4	8.8	5.2
BSA	Day 0	3.0	1.5	9.75
	Day 56	8.5	2.5	9.0
PGA	Day 0	2	2	2
	Day 56	2	3	2

moderate chronic plaque psoriasis for ≥ 12 months prior to the first dose of the study drug. Patients were required to have a Psoriasis Area and Severity Index (PASI) score ≤ 12 , a Physician's Global Assessment (PGA) score of 2 or 3, and $<10\%$ Body Surface Area (BSA) psoriasis involvement (Table 1). Patients were required to have adequate haematological, renal, and hepatic function.

Genistein dosing, skin biopsies and blood sampling. Genistein dosing and blood and skin biopsy preparation for gene profile evaluation: Patients were screened 1–21 days prior to the administration of the first dose of genistein or placebo on day 0. They were enrolled in this multicenter, double-blind, placebo-controlled study, randomized (1:1) to treat with oral film-coated tablets in one of two treatment regimens: (1) genistein 75 mg or 150 mg per day or (2) placebo. Genistein (GEN) was synthesised at the Pharmaceutical Research Institute (Warsaw, Poland) (Table 1).

Samples of blood and skin biopsy were collected at two time points: baseline (Day 0) and 56 days (Day 56) of treatment. Biopsies were taken under local anaesthesia from 4 psoriatic participants. One 3 mm punch biopsy was obtained from psoriatic plaque skin (PP) and the other psoriatic normal skin (PN), sampled at least 10 cm away from any active plaque. Venous blood for gene expression profiling was collected by phlebotomy with a butterfly needle into PAXgene tubes. The first drawn tube from all patients was ejected, as suggested by the PAXgene procedure.

RNA processing

Skin biopsies. After removal from the skin, biopsies were snap-frozen in liquid nitrogen and stored at -80°C until use. The isolation of total RNA from psoriatic plaque and psoriatic normal skin (unaffected skin) biopsies was carried out using an RNA Fibrous Tissues Mini Kit (Qiagen, Germany) and Trizol (Ambion, USA), according to the manufacturer's protocol. **Blood.** Total RNA was extracted from the whole blood of patients and control subjects using the PAXgene Blood RNA Kit (Qiagen, Germany) according to the manufacturer's protocol. Analysis of RNA quality and quantity was performed using the Nanodrop (ThermoFisher Scientific,

USA) and RNA 6000 Nano Assay on the Agilent 2100 Bioanalyser (Agilent Technologies, USA).

Real-time quantitative reverse transcription PCR (real-time qRT-PCR)

The reverse transcription reaction was performed on an RNA template and cDNA was synthesised using the Transcriptor First-Strand cDNA Synthesis Kit (Roche Applied Science, USA) according to the manufacturer's protocols. All probes for real-time qRT-PCR are summarised in Table 2. The 63 genes were selected on the basis of the classification criteria referring to the most up-to-date scientific reports describing a list of genes with significantly differentiated activity obtained in both *in vitro* and *in vivo* analyses when studying psoriatic phenotypes, stated at least a couple of times in unrelated literature sources (Bocheńska *et al.*, 2017; Coda *et al.*, 2012; Emmerson *et al.*, 2010; Galluzzo *et al.*, 2018; González *et al.*, 2011; Gudjonsson *et al.*, 2009; Gudjonsson *et al.*, 2010; Martin *et al.*, 2013; Tian *et al.*, 2012). Except for published lists of genes, we also studied and reviewed the psoriasis-associated genes database (PAGD), as well as the database of genes associated with psoriasis (db-GAP). Thus, applied real-time qRT-PCR assays were obtained for genes encoding proteins involved in lipid metabolism, antimicrobial defences, epidermal differentiation and the control of cutaneous vasculature. As keratinocytes at inflammatory sites of the epidermis induce the abnormal expression of cytokines and chemokines, leading to the infiltration of T cells or leukocytes (Tian *et al.*, 2012; Dowlatsahi *et al.*, 2013), we also examined the effects of genistein on the expression of selected chemokine genes.

The pre-plated PCR assays contained target specific primers and a matching probe from the Universal Probe Library (UPL). Real-time qRT-PCR was run using the Light Cycler 480 II detection system (Roche Applied Science, USA) and the quantification of gene expression was determined by the comparative $\Delta\Delta\text{Ct}$ method. The analysis of transcripts driven from the tested genes showed those in which expression was identified as being different by at least 0.7- and 1.3-fold changes ($p < 0.05$): (skin samples) in lesional involved psoriatic

Table 2. Sequences of primer and probe numbers applied for real-time qRT-PCR.

Gene symbol	Forward sequence	Reverse sequence	UPL probe no.	Skin	PBCs
ACACB	GGGAGAGAACCGGAAGA	CCTCGGATGGACAGTTCCT	77	‡	
AKT1	GCAGCACGTGTACGAGAAGA	GGTGTCACTCTCCGACGTG	45		‡
CAT	CGCAGTTCGGTTCTCCAC	GGGTCCCGAACTGTGTCA	67	‡	
CCL2	AGTCTCTGCCGCCCTTCT	GTGACTGGGGCATTGATTG	83		‡
CCL3	GCTGACTACTTTGAGACGAGCA	GCTTCGCTTGGTTAGGAAGA	125		‡
CCL4	TGCTTCTCCATGAGACACATCT	AAAGATTAAATTAAGAGAAGGGACAGG	55	‡	‡
CCL5	CCTCATTGTACTGCCCCTCT	GGTGTGGTGTCCGAGGAATA	16	‡	‡
CCL19	CACTACCTTCTCATCAAGGATGG	CCCCTCAGTGTGGTGAACA	40		‡
CXCL1	CATCGAAAAGATGCTGAACAGT	CTTCAGGAACAGCCACCAGT	52	‡	‡
CXCL6	CACGAGGAAACCAAGTGC	GGGAGGCTCATAGTGGTCAA	65		‡
CXCL10	AAAAGGTATGCAATCAAATCTGC	AAGAATTTGGGCCCTTG	86	‡	‡
DEFB4A	TCAGCCATGAGGGTCTTGTA	GGATCGCTATACCACCAAA	35	‡	‡
EGFR	CAGCCACCCATATGTACCATC	AACTTTGGGCGACTATCTGC	42		‡
FABP5	GCAGACCCCTCTCTGCAC	TCGCAAAGCTATTCCTACTC	11	‡	
FADS1	CATCAACATGCATCCCTTCTT	TTTGTGCTGGTGGTTGTACG	60	‡	
FOSL1	AGGAACTGACCGACTTCCTG	TCTGCAGTCTCTCAATCTCTC	26	‡	
FOXC1	TGCTTTTCAGAGACCTGCTTT	GCAAGGAAGAAGGCAAGAGA	52	‡	
FOXO1	GCAGCCAGGCATCTCATAAC	GCATGTCCAGGGTGGGTAT	33	‡	
GATA3	CTCATTAAGCCCAAGCGAAG	GTCTGACAGTTCGCACAGGA	108		‡
GJB2	CAAGAACGTGTGCTACGATCA	GCGTGGACACGAAGATCAG	60	‡	
IL1A	GAATGACGCCCTCAATCAAA	TGATGACTTATAAGCACCCATGTC	66	‡	‡
IL1B	AGCTGATGGCCCTAAACAGA	TCGGAGATTCGTAGCTGGAT	85	‡	‡
IL1RN	TGCCTGTCTGTGTCAAGTC	TCTCGCTCAGGTCACTGATG	54	‡	‡
IL2	TGTTCAAGAGTTCCTATCACTCTC	GCAAGACAGGAGTTGCATCC	51		‡
IL6	ACCGGGAACGAAAGAGAAG	GAAGGCAACTGGACCGAAG	133	‡	‡
IL8	TAGCCAGGATCCACAAGTCC	CTGTGAGGTAAGATGGTGGCTA	98	‡	‡
IL10	CTGCCTAACATGCTTCGAGA	GTCCAGCTGATCCTTCATTG	161	‡	‡
IL11	AGCTGCAAGGTCAAGATGGT	AGATTGTTTCCAGTTTGTATGG	76		‡
IL12A	CACTCCCAAAACCTGCTGAG	TCTCTTCAGAAGTGCAAGGGTA	50	‡	‡
IL17A	CCTATAAGCAAAACAAAGCATGTC	CGAGAACTTGAATTTTGGGTA	85		‡
IL18	AAGGAATTGTCTCCAGTGC	CCTCTCCCAAGCTGTGTA	71	‡	‡
IL20	ACAGCCAGATTCTGAGTCACTTT	AGCAGCATCACTTTCCTCT	25	‡	‡
IL20RA	GCGTACACGTGGAGTCCTTC	AGCCTTGAAGTCTGATGATTGAT	81		‡
IL23A	GAAGTGCTAGGATCGGATATTT	AGTAGGAGGCATGAAGCTG	32	‡	‡
IL24	GCTGCAGCAGGAGGTCT	GCAGGGTGTGGACAAGGTAA	89		‡
JAK1	AATGGCTGTATGGTCCAAT	TACATCCCCTCCTCGCTTC	147	‡	‡
JAK2	CAGGAACAAGATGTGAAGTGTTC	CCCATGCAGAGTCTTTTTCAG	52	‡	‡
JUN	AGGATAGTGCATGTTTCAGG	GACTTCTCAGTGGGCTGTCC	65		‡
KRT6B	GGCCCTGGATGTGGAGAT	TGACTTGTCCAACGCCTTC	67	‡	
MMP10	CAAAAGAGGAGGACTCCAACA	TTCACATCTTTTCGAGGTTG	61		‡
NFKB1	CTGGCAGCTCTTCTCAAAGC	TCCAGGTATAGAGAGGCTCA	68	‡	‡
NFKBIZ	TTGATACCATTAAAGTGCCTAATTCA	TTCTTCAGCTGCCAAATGC	32		‡
NOTCH1	TCAATGAGTTCAGTGCGAGT	GCACACTCGTCCACATCGTA	105		‡
PCYOX1	GCTCCGCCAGATAAAATCG	CAAATTTCTGCCGAGGT	66	‡	

PI3	AGCCAGTCAAAGGTCCAGTC	GCGCACCGGATCAAGATA	51	‡	‡
RHOB	CCCCTGAGCATGCTTTTCT	CGAGGGGAGTCGAACAGAC	5	‡	
RPLP0	TCGACAATGGCAGCATCTAC	GCCAATCTGCAGACAGACAC	6	‡	‡
SERPINB3	CAACAAGCTCTTCGGAGAAAA	TGGAGCATTGCAAAATCAA	147	‡	
SIGIRR	CTGGTGACCTTGCTGCTCT	TGCACCTCTTTCCAAAATCG	12	‡	‡
SOCS1	GGTCCCCCTGGTTGTTGTA	GTAGGAGGTGCGAGTTCAGG	36	‡	‡
SOCS2	AGGCCTCACTGCAATTGAT	TGCAAAATATAAAATGCCCAAG	40		‡
SP1	CTATAGCAAATGCCCCAGGT	TCCACCTGCTGTGTCATCAT	131	‡	‡
STAT1	GGATTGAAAGCATCTAGAACTCA	GATGAAGCCCATGATGCAC	32		‡
STAT3	TCCTGAAGCTGACCCAGGTA	GGTCGTTGGTGTCACACAGAT	43	‡	‡
STAT4	ATCTGCCTCTATGGCCTGAC	AATCATCACCACAGGCAATG	54		‡
SYNCRIP	CGATACCATCGGACAGGATT	TCTGTAGCCATGTTCCAGAGAT	30	‡	
TLR1	AGGGGACAATCCATTCCAA	TTGGTCTATATTTTGACAAATTCTCC	100	‡	‡
TLR4	CAAGATGCCCTTCCATTT	TCCTTAGGAATTAGCCACTAGACTTT	94		‡
TNF	CGGTGCTTGTCTCAGC	GCCAGAGGGCTGATTAGAGA	12		‡
TUFT1	GGAAAGTCCGGCAAATGATA	GCGTCCTTGACTGGATCAC	50	‡	
UST	TGTGGACAGCATCCAGAT	TCATTACAGTTCAGCTTTGC	116	‡	
YWHAZ	GATCCCCAATGCTTCACAAG	TGCTGTGTGACTGATCGAC	102	‡	‡
ZNF12	GATCTGCCTCCGCTTTCAC	ACAGCCACGTCCTTGAATG	65	‡	
ZNF483	ACTGGAAAACCTCAGGAACCTA	CATGGCAATTC AACCCACT	33	‡	

‡points the analysis of a given gene in the skin or the PBC' samples.

skin (PP_D0) *vs.* uninvolved psoriatic skin (PN_D0) of u.09, 11, 12 and 15 patients on day 0 (baseline). Also, lesions involved the psoriatic skin of patients following 56 days (PP_D56) of 75 mg (u.09 and 12 patients) or 150 mg (u.15 patient) genistein treatment handling or placebo (u.11 patient) *vs.* baseline (PP_D0); (blood samples) in u.09, 11, 12 and 15 patient's PBCs *vs.* normal healthy people (control group) on day 0 (baseline), and in PBCs of patients following 56 days of 75 mg (u.09 and 12 patients) or 150 mg (u.15 patient) genistein treatment handling or placebo (u.11 patient) *vs.* baseline. Results were normalised to the expression of housekeeping genes such as *RPLP0* (ribosomal protein lateral stalk subunit P0) and *YWHAZ* (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta).

RESULTS

Alterations in gene expression in lesional psoriatic skin

In summary, we have analysed the activity of the 41 genes indicated in Table 2. Due to the limited amount of isolated RNA from patient samples, studies on $n=1$ were performed. In general, in the 4 paired PP (lesional involved psoriatic skin (PP_D0)) and PN (uninvolved psoriatic skin (PN_D0)) skin sections from 4 patients (u.09, 11, 12 and 15) on day 0 (baseline), a rather clear separation of the PN_D0 samples from the PP_D0 samples was revealed (Table 3). On the basis of our criteria for differentially regulated transcripts, we identified that all 41 genes differed in expression between the PP_D0 and PN_D0 samples, at least for one reference (*RPLP0* or *YWHAZ*) and minimum for the individual patient from the 4 subjects, analysed by real-time qRT-PCR (Table 3). Most of these genes (21 in total) were either down- or

up-regulated, or unchanged, or had no data available, depending on the patient being analysed. Another 18 mRNAs revealed up-regulated or unchanged records, or no data accessible, again depending on the subject being studied. Similarly, the activity of two other genes, namely *FADS1* and *TUFT1*, was decreased or remained unaffected depending on the patient being tested. Seven of the 41 modulated genes, visible among all four subjects, gained an increase in expression of as much as 10 to 20 times. These included genes coding for the C-C motif chemokine ligand 4 (*CCL4*), anti-microbial peptides, including human β -defensin 2 (*DEFB4*), and transcription factor forkhead box protein O1A (*FOXO1*), interleukin (IL) family members, such as interleukin 1B (*IL1B*) and 8 (*IL8*), peptidase inhibitor 3 (*PI3*) and serpin family B member 3 (*SERPINB3*).

Genistein-responsive genes in skin biopsies

To investigate the impact of genistein on the gene activity profile of inflamed skin (PP), we compared the gene expression of skin biopsy samples at baseline (PP_D0) and after 56 days (PP_D56) of therapy in the four patients, i.e. placebo, 75 or 150 mg genistein-treated. Again, as in the previous experiment, we also analysed the expression of 41 genes (Table 2), with $n=1$ only due to the restricted access to a larger quantity of material, with respect to reference genes, *RPLP0* and *YWHAZ* at a constant expression level, by using real-time qRT-PCR. Response to genistein was associated with a significant expression change in all 41 of the studied genes, irrespective of the patient, and for at least one reference (Table 3). As in the case of transcripts that differed in level between the PP_D0 and PN_D0 samples, a differentiated profile of gene activity modulation was also observed, depending on the examined patient. In gen-

Table 3. Alterations in the mRNA level of 41 studied genes in lesional involved psoriatic skin (PP_D0) vs. uninvolved psoriatic skin (PN_D0) in patients named u.09, 11, 12 and 15, on day 0 (baseline). In addition, changes in the activity of those 41 analysed genes in lesional biopsies from psoriatic skin at baseline (PP_D0) and after 56 days (PP_D56) of 75 (u.09 and 12 patients) or 150 mg (u.15 patient) genistein treatment or placebo (u.11 patient). The deregulation of genes was referred to as 0.72FC \geq 1.3, and with respect to reference genes, *RPLP0* or *YWHAZ* of constant expression level, studied with the use of real-time qRT-PCR. Due to the limited amount of isolated RNA from patient samples, studies on $n=1$ were performed. The values representing the deregulation of particular genes in individual patients are shown in bold, i.e. between the PP_D0 and PN_D0 samples, and between the PP_D56 and PP_D0 samples, respectively.

Gene symbol	Fold change						u.11 (placebo)						u.12 (75 mg genistein)						u.15 (150 mg genistein)					
	u.09 (75 mg genistein)			u.11 (placebo)			u.12 (75 mg genistein)			u.15 (150 mg genistein)			u.12 (75 mg genistein)			u.15 (150 mg genistein)			u.12 (75 mg genistein)			u.15 (150 mg genistein)		
	PP_D0 vs. PN_D0	PP_D56 vs. PP_D0	RPLP0	YWHAZ	PP_D0 vs. PN_D0	PP_D56 vs. PP_D0	RPLP0	YWHAZ	PP_D0 vs. PN_D0	PP_D56 vs. PP_D0	RPLP0	YWHAZ	PP_D0 vs. PN_D0	PP_D56 vs. PP_D0	RPLP0	YWHAZ	PP_D0 vs. PN_D0	PP_D56 vs. PP_D0	RPLP0	YWHAZ	PP_D0 vs. PN_D0	PP_D56 vs. PP_D0	RPLP0	YWHAZ
ACACB	2.8	3.1	0.2	0.2	0.2	0.2	0.05	0.04	0.8	0.8	0.8	0.8	0.7	0.6	1.3	2.0	n.d.	n.d.	n.d.	n.d.	n.d.	0.7	0.7	0.6
CAT	1.2	1.4	0.4	0.4	0.4	0.1	0.1	0.1	0.9	0.8	0.8	0.8	0.9	0.8	1.4	2.2	n.d.	n.d.	n.d.	n.d.	n.d.	1.0	0.9	0.9
CCL4	4.3	4.9	0.4	0.3	0.3	2.5	2.5	2.5	1.3	1.3	1.3	1.3	n.d.	n.d.	0.5	0.5	12.1	13.2	0.6	0.6	0.6	0.6	0.6	0.6
CCL5	1.8	2.1	0.4	0.4	0.4	0.5	0.5	0.5	1.2	1.2	1.2	1.2	n.d.	n.d.	0.5	0.5	1.5	1.6	1.2	1.0	1.0	1.0	1.0	1.0
CXCL1	2.3	2.6	0.6	0.5	0.5	2.7	2.7	2.7	1.5	1.5	1.5	1.5	n.d.	n.d.	0.4	0.4	n.d.	n.d.	n.d.	0.6	0.6	0.6	0.6	0.6
CXCL10	1.6	1.8	0.9	0.7	0.7	3.8	3.8	3.8	1.1	1.0	1.0	1.0	n.d.	n.d.	0.5	0.5	2.4	2.6	1.4	1.2	1.2	1.4	1.2	1.2
DEFB4A	0.8	0.9	1.9	1.6	1.6	14.0	14.0	14.2	1.3	1.3	1.3	1.3	n.d.	n.d.	0.6	0.6	6.3	6.8	1.6	1.5	1.5	1.6	1.5	1.5
FABP5	2.3	2.6	0.9	0.9	0.9	3.2	2.9	2.9	0.9	0.9	0.9	0.9	3.2	2.9	1.5	2.3	n.d.	n.d.	n.d.	0.9	0.7	0.9	0.7	0.7
FADS1	1.1	1.2	0.6	0.5	0.5	0.3	0.2	0.2	1.2	1.2	1.2	1.2	0.7	0.6	1.2	1.8	n.d.	n.d.	n.d.	0.7	0.6	0.7	0.6	0.6
FOSL1	1.5	1.7	1.7	1.7	1.7	2.2	1.9	1.9	1.1	1.1	1.1	1.1	6.5	5.8	0.6	0.9	n.d.	n.d.	n.d.	0.9	0.8	0.9	0.8	0.8
FOXC1	1.5	1.7	0.4	0.4	0.4	0.2	0.2	0.2	1.1	1.1	1.1	1.1	0.4	0.3	1.1	1.7	n.d.	n.d.	n.d.	1.2	1.1	1.2	1.1	1.1
FOXO1	8.7	22.8	0.4	0.4	0.4	0.2	0.2	0.2	1.0	1.0	1.0	1.0	7.8	7.0	0.9	1.4	17.0	18.4	1.0	0.8	0.8	1.0	0.8	0.8
GJB2	1.4	1.5	0.9	0.9	0.9	1.9	1.7	1.7	1.2	1.2	1.2	1.2	2.3	2.0	0.7	1.1	n.d.	n.d.	0.8	0.7	0.7	0.8	0.7	0.7
IL1A	1.8	2.0	0.5	0.4	0.4	1.9	1.9	1.9	1.6	1.6	1.6	1.6	n.d.	n.d.	0.7	0.7	6.5	7.1	0.3	0.3	0.3	0.3	0.3	0.3
IL1B	2.6	2.9	0.3	0.2	0.2	1.9	1.9	1.9	1.0	1.0	1.0	1.0	n.d.	n.d.	1.2	1.1	17.3	18.8	0.2	0.2	0.2	0.2	0.2	0.2
IL1RN	1.2	1.4	0.7	0.6	0.6	1.0	1.1	1.1	1.3	1.3	1.3	1.3	n.d.	n.d.	1.5	1.4	2.4	2.6	1.1	0.9	0.9	1.1	0.9	0.9
IL6	0.8	0.9	0.5	0.4	0.4	0.9	0.9	0.9	1.7	1.7	1.7	1.7	n.d.	n.d.	0.5	0.5	1.4	1.5	0.4	0.3	0.3	0.4	0.3	0.3
IL8	3.4	3.9	0.4	0.3	0.3	4.6	4.7	4.7	0.7	0.7	0.7	0.7	n.d.	n.d.	5.9	5.7	22.0	24.0	0.2	0.2	0.2	0.2	0.2	0.2
IL10	4.5	5.1	0.3	0.2	0.2	1.6	1.7	1.7	1.1	1.1	1.1	1.1	n.d.	n.d.	0.7	0.6	2.5	2.7	1.9	1.7	1.7	1.9	1.7	1.7
IL12A	6.6	7.5	0.1	0.1	0.1	n.d.	n.d.	n.d.	2.4	2.4	2.4	2.4	n.d.	n.d.	n.d.	n.d.	2.1	2.3	0.1	0.1	0.1	0.1	0.1	0.1
IL18	1.5	1.7	0.4	0.3	0.3	0.2	0.2	0.2	1.0	1.0	1.0	1.0	n.d.	n.d.	2.0	2.0	1.9	2.0	1.0	0.9	0.9	1.0	0.9	0.9
IL20	2.1	2.3	0.5	0.4	0.4	2.3	2.3	2.3	2.0	2.0	2.0	2.0	n.d.	n.d.	0.5	0.5	n.d.	n.d.	1.0	0.9	0.9	1.0	0.9	0.9
IL23A	1.6	1.8	0.3	0.3	0.3	0.3	0.3	0.3	1.6	1.5	1.5	1.5	n.d.	n.d.	1.4	1.4	1.3	1.5	1.2	1.0	1.0	1.2	1.0	1.0
JAK1	1.2	1.4	0.4	0.3	0.3	0.2	0.2	0.2	1.0	1.0	1.0	1.0	n.d.	n.d.	1.1	1.1	1.2	1.3	0.9	0.8	0.8	0.9	0.8	0.8

JAK2	2.7	3.1	0.3	0.2	0.2	0.2	0.7	0.7	n.d.	n.d.	1.0	0.9	n.d.	n.d.	0.9	0.8
KRT6B	0.8	0.9	1.4	1.3	5.1	4.5	1.1	1.1	3.2	2.9	0.5	0.8	3.6	3.9	0.6	0.5
NFKB1	1.7	1.9	0.3	0.3	0.3	0.3	1.1	1.1	n.d.	n.d.	1.1	1.1	3.7	4.0	1.0	0.9
PCYOX1	2.2	2.5	0.5	0.4	0.2	0.2	1.0	1.0	0.5	0.4	1.7	2.6	n.d.	n.d.	0.9	0.7
PI3	1.0	1.2	1.5	1.4	5.1	4.5	1.4	1.3	16.3	14.7	0.5	0.8	n.d.	n.d.	0.8	0.7
RHOB	1.3	1.5	0.5	0.5	0.1	0.1	1.1	1.0	0.4	0.3	1.8	2.8	n.d.	n.d.	1.1	0.9
SERPINB3	3.4	3.8	0.7	0.7	5.4	4.7	1.2	1.1	13.8	12.4	0.8	1.2	n.d.	n.d.	1.0	0.9
SIGIRR	1.9	2.2	0.3	0.3	0.1	0.1	0.9	0.9	n.d.	n.d.	0.6	0.6	0.6	0.7	0.7	0.7
SOC51	2.9	3.3	0.4	0.3	2.0	2.0	1.4	1.4	n.d.	n.d.	0.4	0.4	4.8	5.2	1.0	0.8
SP1	1.5	1.7	0.4	0.3	0.1	0.1	1.0	1.0	n.d.	n.d.	1.4	1.4	1.1	1.2	1.0	0.9
STAT3	2.5	2.8	0.5	0.4	0.6	0.6	1.2	1.2	n.d.	n.d.	1.1	1.1	5.7	6.2	0.9	0.8
SYNCRIP	1.2	3.7	0.5	0.5	0.4	0.3	0.8	0.8	1.0	0.9	1.0	1.6	n.d.	n.d.	0.5	0.4
TLR1	2.2	2.5	0.3	0.3	0.2	0.2	0.8	0.8	n.d.	n.d.	0.9	0.9	1.2	1.3	1.1	0.9
TUFT1	1.0	1.2	0.6	0.6	0.2	0.2	1.0	1.0	0.4	0.4	1.5	2.4	1.1	1.1	1.0	0.9
UST	2.4	2.7	0.3	0.3	0.1	0.1	1.5	1.4	0.5	0.5	1.2	1.8	1.1	1.2	1.1	0.9
ZNF12	1.4	1.6	0.5	0.4	0.2	0.2	0.8	0.8	1.1	1.0	1.1	1.7	2.5	2.7	0.7	0.6
ZNF483	6.8	7.7	0.2	0.2	0.2	0.2	0.9	0.9	1.2	1.1	1.6	2.4	n.d.	n.d.	0.4	0.4

n.d. shortcut stands for no data available.

eral, the smallest number of deregulated genes was identified for placebo (u.11). There were 14 genes in total, 12 with increased and 2 with decreased expression. Studying carefully the gene expression levels modulated in placebo, we found that stimulated *CCL4*, *CXCL10*, *IL1A* and *IL6* were down-regulated for all three patients treated with genistein. The other ten genes with modulated activity in placebo exhibited a substantially varied pattern of expression in individual patients treated with genistein. In turn, the highest number of deregulated genes was observed for patient u.09, where all of the studied mRNAs, except for *FABP5* and *GJB2*, revealed a change in the level at PP_D56 *vs.* PP_D0. Furthermore, in the case of both doses of 75 (u.09) and 150 (u.15) mg genistein-treated patients (PP_D56 *vs.* PP_D0), most of the genes were down-regulated and only few showed accelerated activity once exposed to the tested isoflavone. However, the opposite trend was found for patient 12; that is, with a significant majority of up-regulated genes. In addition, the most significantly deregulated among all three patients in response to genistein (PP_D56 *vs.* PP_D0) was *IL8*, depending on the subject, from 3 to 5 times, exhibiting either a decrease in mRNA level (for u.09 and 15, respectively) or an increase (around 6 times) in transcript amount (for u.12).

It should be noted that all 14 of the deregulated genes in u.11 placebo were found among the 38 transcripts (while for *IL12A* transcript we had no data available) which differed in level between the PP_D0 and PN_D0 samples (Table 3). Likewise, for genistein-treated patients, we identified a number of genes in a set of the transcripts that differed in levels between the PP_D0 and PN_D0 samples. Correspondingly, we found that most of the genes (except for *DEFB4A*, *FOSL1*, *KRT6B* and *PI3* in case of u.09; and *SIGIRR*, *SP1*, *TLR1*, *TUFT1* and *UST* in case of u.15), being deregulated in PP *vs.* PN on day 0, showed a considerable reduction in activity after genistein handling (D56). For u.12, we found 11 of the 18 genes with modulated activity in the PP_D0 *vs.* PN_D0 were stimulated in response to genistein (PP_D56 *vs.* PP_D0).

Changes in gene activity in the PBCs of psoriatic patients

To identify genes that were differentially regulated in the PBCs of u.09, 11, 12 and 15 psoriatic patients, we examined the expression profile of the 45 genes listed in Table 2. In total, 16 out of 45 transcripts that differed in level between the psoriatic patients' and were varied from normal healthy control PBCs, in respect to *RPL-PO* reference, and minimum for the individual patient from the 4 subjects, analysed

Table 4. List of 16 deregulated genes of the 45 studied in psoriatic PBCs, i.e. genes differentially expressed between u.09, 11, 12 and 15 psoriatic patients and the normal healthy people on day 0 (baseline), analysed via real-time qRT-PCR. Alterations in transcript amount of the selected genes relate to an expression ratio greater than or equal to 1.3-fold and below or equal to 0.7, and with respect to *RPLPO* mRNA expression at a constant level. Due to the limited amount of isolated RNA from patient samples, studies on $n=1$ were performed.

Gene symbol	Fold change			
	u.09 (75 mg genistein)	u.11 (placebo)	u.12 (75 mg genistein)	u.15 (150 mg genistein)
CCL2	0.5	0.5	0.2	0.8
CCL5	0.6	0.7	0.7	0.9
CXCL6	1.6	2.6	2.6	n.d.
CXCL10	1.8	2.2	3.5	4.6
IL1A	0.9	1.9	0.9	1.3
IL2	2.7	n.d.	4.4	n.d.
IL6	0.6	1.2	1.8	2.4
IL8	2.6	1.4	1.7	6.1
IL10	0.6	1.1	0.1	0.5
IL11	1.0	4.6	6.2	8.7
IL12A	0.4	0.4	0.2	0.9
IL17A	0.4	1.7	4.3	n.d.
JUN	1.0	1.8	2.4	5.4
SOC2	0.6	0.7	0.8	0.8
STAT4	0.7	0.5	0.7	1.0
TLR1	0.9	1.5	0.7	2.6

n.d. shortcut stands for no data available.

by real-time qRT-PCR were affected (Table 4). Due to the limited amount of isolated RNA from patient samples, studies of $n=1$ were performed. In common between the four psoriatic patients when studying their PBC expression profiles, two genes revealed increased activity (i.e. *CXCL10* and *IL8*, coding for the C-X-C motif chemokine ligand 10 and interleukin 8, respectively), while another two were down-regulated (*CCL2* and *CCL5*, correspondingly coding for the C-C motif chemokine ligand 2 and C-C motif chemokine ligand 5, respectively) (Table 4). The largest number of genes with modulated activity (14 genes) was observed for patient u.12 (treated with 75 mg genistein). Among them 8 genes were up-regulated, as is the case for subject u.11. In turn, the smallest number of genes (i.e. 8) with the only single down-regulated gene had u.15 subject (taking 150 mg genistein).

Modulated transcripts' level in the PBCs in response to genistein

To study the effect of genistein on the gene expression profile of PBCs from psoriatic subjects treated with 75 mg (u.09 and 12 patients) and 150 mg (u.15 patient) genistein or placebo (u.11 patient), we compared the mRNA levels at baseline (day 0) and after 56 days of therapy with respect to housekeeping *RPLPO* and *YWHAZ* genes and by real-time qRT-PCR (Fig. 1). Once more, as in the previous experiment, we also analysed the expression of 45 genes in total (Table 2), with $n = 1$ only due to the restricted access to a larger quantity of material. The analysis revealed significant alterations in the activity of 36 of the 45 genes (Fig. 1). Then, we realised that there were 15 transcripts among those 36 genes (except for *SOC2*) that differed in levels between

the psoriatic patient PBCs and those derived from normal healthy controls.

Wherein the largest number of genes (i.e. 23) with modulated activity was for u.11 (with 21 up-regulated and 2 down-regulated), the fewest genes (i.e. 7) were reported for patient u.15 (Fig. 1). Of the 36 genes with modulated activity, only two, *CCL2* and *IL10*, were down-regulated, albeit diversified, in all 4 subjects tested. The most significantly stimulated were genes in patients treated with 75 mg genistein (i.e., *IL17A* app. 6-fold for u.09 and *IL10* app. 8-fold for u.12). On the other hand, the most considerably, as much as 3 to 6 times, down-regulated were *IL6*, *IL11*, *IL17A* and *CXCL6* genes, with patient u.12 taking 150 mg genistein.

It is worth adding that, as in the case of gene activity analyses in skin specimens, where it was possible to determine a set of common genes between those with modulated activity in patients entering genistein tests and those that differed in level between the PP_D0 and PN_D0 samples, a similar circumstance was observed in PBC studies. Thus, 7 of the 23 genes with modulated activity in u.11 placebo (Fig. 1) were found among the 11 transcripts that differed in levels between the psoriatic patients' and derived from normal healthy control PBCs (Table 4). Similarly, for genistein-treated patients we identified a number of genes in a set of the transcripts that differed in level between the PBC of psoriatic patients and normal healthy controls. Respectively, we found 7 of the 16 genes to have modulated activity in the case of u.09 (Fig. 1) from 11 transcripts (Table 4), 11 of the 20 genes with modulated activity in the case of u.12 (Fig. 1) from 14 transcripts (Table 4), and 2 of the 7 genes with modulated activity present in u.15 (Fig. 1) from 8 transcripts (Table 4). Remarkably, our results showed that the aberrant expression of some of these

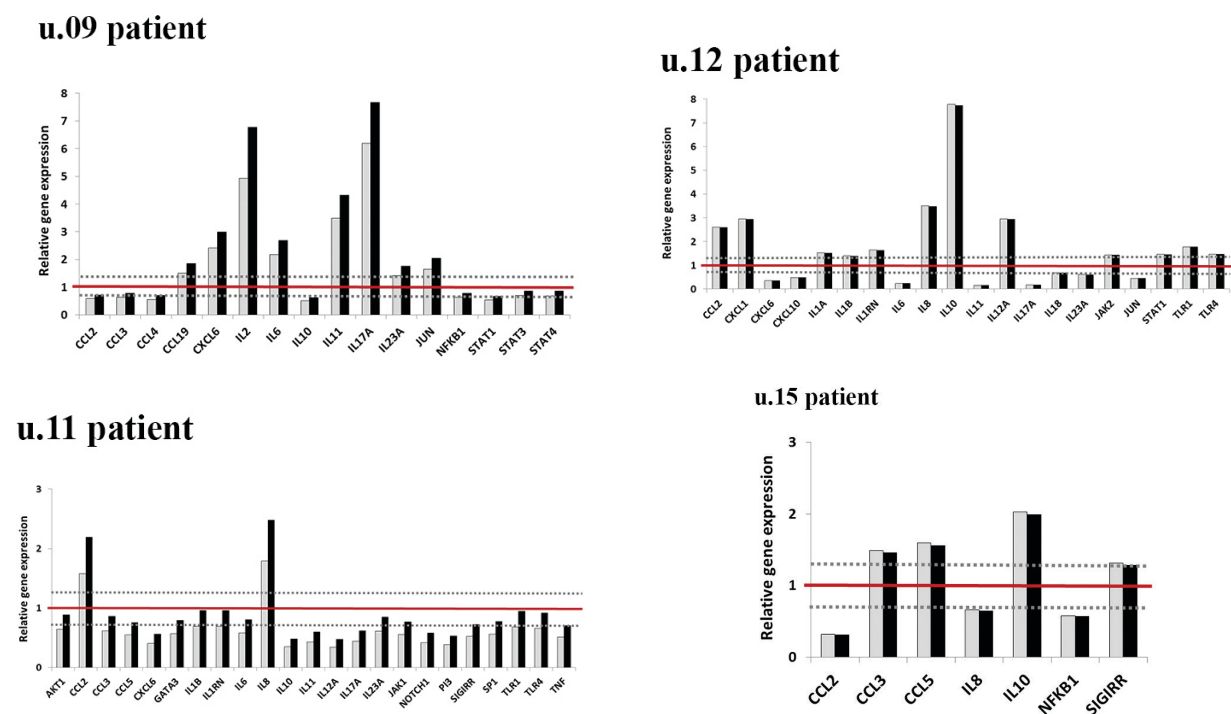


Figure 1. Alterations in the activity of 36 genes in PBCs from psoriatic subjects:

treated with 75 mg (u.09 and 12 patients) and 150 mg (u.15 patient) genistein or placebo (u.11 patient) on Day 56 vs. Day 0 (baseline, red line), analysed with the use of real-time qRT-PCR are referred to as $0.7 \geq FC \geq 1.3$ (grey lines). The results are shown relative to the expression of the housekeeping genes RPLPO (grey bars) and YWHAZ (black bars). Due to the limited amount of isolated RNA from patient samples, studies on $n=1$ were performed.

genes affected in psoriasis (Table 4) are improved by the action of genistein (Fig. 1).

Deregulated profile of gene expression in the skin specimens and the PBCs in response to genistein

Also noteworthy is the comparison of gene activity in the skin specimens and PBCs of u.09, 11, 12 and 15 patients participating in the genistein test (Fig. 2). Because the PBC analysis was basically representative of inflammation-immune axis regulation genes, this comparison is just about these mRNAs. It was possible to produce a refined list of genes that were consistently differentially expressed in both PP skin and PBCs derived from all three patients treated with genistein, while not for placebo. Within this list, there are several genes that were deregulated; for patient u.09 we found 4 down-regulated transcripts (*CCL4*, *IL10*, *NFKB1* and *STAT3*), for subject u.12 there were 2 decreased (*CXCL10* and *IL6*) and 1 stimulated in activity (*IL1RN*), and in the case of patient u.15, there was one with increased (*IL8*) and one reduced (*IL10*) mRNA level.

DISCUSSION

Various topical medications, phototherapy, and systemic drugs are available to treat patients with psoriasis of varying disease severity. However, many studies are constantly performed to detect new therapeutic anti-psoriatic substances, which are verified in preclinical laboratory tests and clinical phase trials (Bocheńska *et al.*, 2017). This includes natural compounds which, as it turns out, are important in the treatment of psoriasis. They are the starting point for further analyses to improve the health and quality of patients' lives. Genistein

turn out to be very important here. For example, Ito's group investigated the topical application of Glyteer (GL, soybean) on a psoriatic model in mice (Ito *et al.*, 1992). Also, topical genistein was found to decrease psoralen-ultraviolet A (PUVA)-induced skin thickening and greatly reduce cutaneous erythema and ulceration in a dose-dependent manner (Shyong *et al.*, 2002). Genistein, a soy-derived molecule has attracted attention as a potent agent in the treatment of psoriasis, not only due to its anti-proliferative and immunosuppressive properties, but also as a mediator modulating the expression of various genes, whose products are involved among others in different phases of inflammation and proliferation (Smolińska *et al.*, 2018). These statements resulted in proposing the performance of test with the use of genistein for monitoring the activity of genes in the material derived from psoriatic patients.

Our study was designed to evaluate the impact of two dosing regimens of genistein at 75 or 150 mg per day administered orally on gene expression in patients with active mild to moderate chronic plaque psoriasis.

The exploratory objectives of this study were to evaluate gene expression levels in the four patients, i.e. placebo (u.11), 75 (u.09 and 12) or 150 (u.15) mg genistein-treated. In total, with the criteria referred to in the Methods section, we selected 64 genes coding for proteins involved in lipid metabolism, antimicrobial defences, epidermal differentiation and control of cutaneous vasculature, as well as immune mediators, for the analyses of 41 transcripts in the skin and 45 mRNAs in PBC samples, correspondingly (Table 2). Indeed, it has been shown in the literature that genistein influences the expression of inflammatory mediators, including the down-regulation of cytokine genes involved in mediating communication between cells (Mirahmadi *et al.*, 2018;

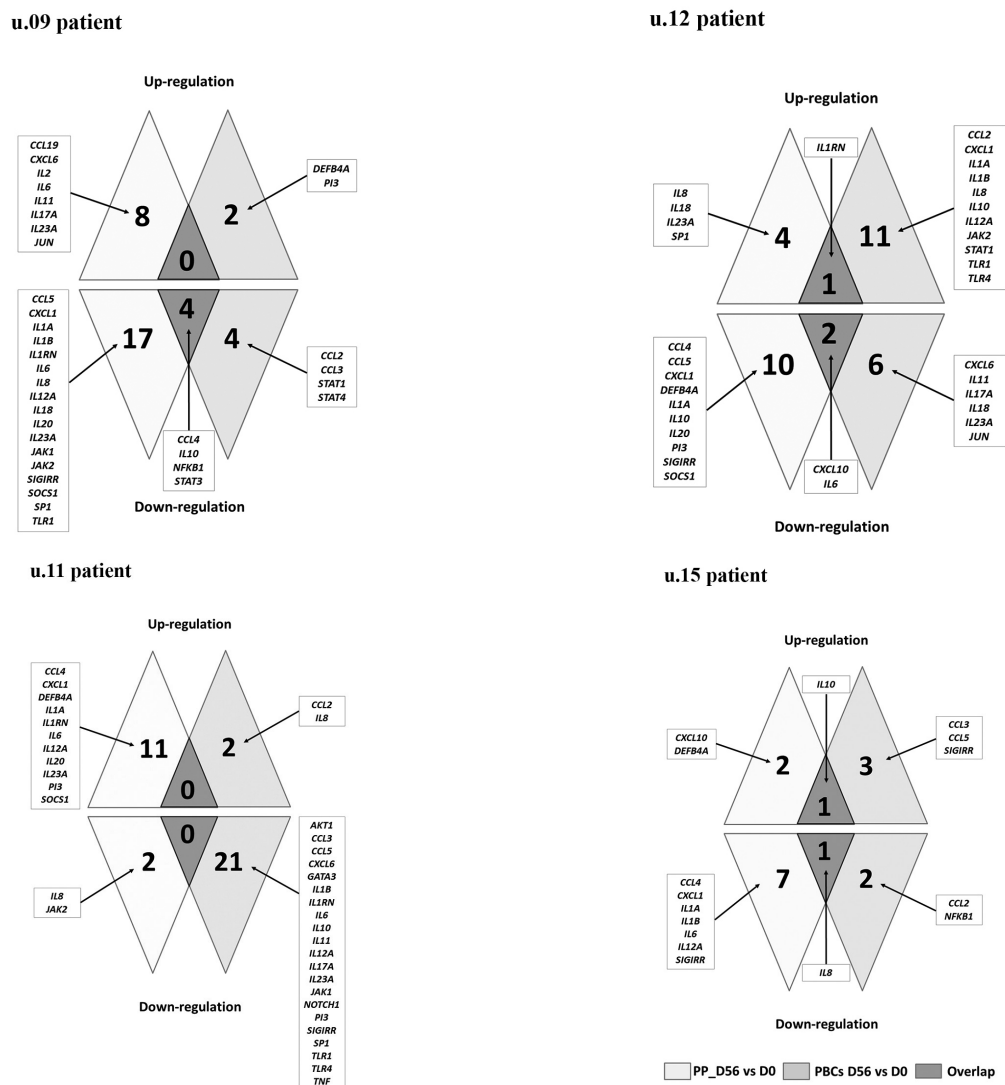


Figure 2. Genes identified as changed under studied conditions with corresponding overlap between the datasets ($0.7 \geq FC \geq 1.3$). Up- and down-regulated genes of the 64 analysed transcripts in lesional involved psoriatic skin (PP) and in peripheral blood cells (PBCs) of patients u.09, 11, 12 and 15 on day 0 (D0), and following 56 days (D56) of genistein treatment or placebo.

Vivatvakin *et al.*, 2017). Initially, however, we dealt with the analysis of gene activity in those four patients before genistein administration. The study was in lesional involved psoriatic skin (PP_D0) *vs.* PN (uninvolved psoriatic skin (PN_D0), and in the PBCs derived from psoriatic patients *vs.* the normal healthy people. The results of these investigations revealed the clear separation of both the PN_D0 samples from the PP_D0 samples, as well as psoriatic PBC samples from the PBCs of normal healthy subjects (Table 3 and 4). In the next step, we compared the gene expression in the same four patients, either treated with 75 or 150 mg genistein, in relation to untreated placebo, again in both of the skin biopsy samples at baseline (PP_D0) and at 56 days (PP_D56) of therapy, and for the PBC samples at day 0 and 56. The outcomes of these works, described in detail in the Results section, allowed significant alterations in gene activity in both skin biopsies and PBCs under the influence of genistein to be noted (Table 3 and Fig. 1). It impairs the activity of certain genes which are over-expressed in psoriasis, while stimulating the expression of other transcripts which are repressed in this dermatosis. Furthermore, the tissue-cross consideration regard-

ing the activity assessment of inflammation-immune axis genes in the material of the skin specimens and PBCs of our four patients joining the genistein test allow us to conclude that this isoflavone exhibits potential as an active anti-inflammatory agent, associated with its capacity to modify the pattern of transcript levels across skin and blood cells (Fig. 2). Also, we found genes that were consistently differentially expressed both in PP skin and PBCs derived from all three patients treated with genistein, while not for placebo.

Moreover, taking into consideration our previous results on the use of “psoriasis-like” HaCaT keratinocytes exposed to isoflavone genistein (Smolińska *et al.*, 2018), we found that the 10 mRNAs (i.e. *CCL4*, *CXCL1*, *CXCL10*, *IL1A*, *IL6*, *IL8*, *KRT6B*, *PI3*, *SIGIRR* and *ZNF483*) studied in psoriatic skin patients were among the genes which were deregulated in the genistein-treated “psoriasis-like” epithelial cell line HaCaT. In turn, when analysing in detail the individual patients, we found 28 transcripts deregulated regarding the modulation profile by genistein in patient u.09, 22 in patient u.12 and 14 in patient u.15, as it was in case of “psoriasis-like” keratinocytes (see Figure 3 in Smolińska *et al.*, 2018).

To the point, we may conclude that, isoflavone genistein, previously seen to be able to modulate the anti-psoriatic members and anti-inflammatory mediators of inflammation, in this study showed some benefits to the aberrant expression of genes contributing to the progression of this dermatosis.

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